GENERAL SESSION II

Discussion Leaders:

DR. MARSHALL R. URIST
DR. FRANKLIN C. MCLEAN

URIST: I am going to ask Dr. Saxén to show some material which does not deal with induction, but before that I would like to ask Dr. Pritchard to make some concluding remarks on the session, "Cellular Differentiation in Bone."

PRITCHARD: Our discussion has centered around the system of cells which lies close to bony surfaces, the cells which are primarily responsible for the formation and resorption of bone matrix and for the production of osteocytes.

The system includes the classic osteoblast and osteoclast and a more generalized type of cell, which used to be called an osteogenic cell or a preosteoblast, but which is now generally referred to as an osteoprogenitor cell. This cell, which is spindle-shaped but otherwise nondescript, was long suspected and recently confirmed to be of the greatest importance in the life of bone. The osteoprogenitor cells are the mother cells which divide to maintain or to increase the population and which differentiate, in suitable circumstances, into osteoblasts, osteoclasts, chondroblasts, and probably fibroblasts. It seems very probable that the reticular cells of bone marrow are really osteoprogenitor cells.

There is some evidence that osteoclasts, and those osteoblasts which do not become osteocytes, may revert to osteoprogenitor cells after their bone-forming and bone-resorbing work is done. This seems to occur in some animals after large doses of parathyroid hormone and in subacute scurvy. On the other hand, there is also evidence that osteoblasts may change to a flat, inactive form—resting osteoblasts—which are later aroused to full functional activity without going through an osteoprogenitor stage. At present, it is difficult to decide whether the flat cells on inactive bone surfaces should be called resting osteoblasts or resting osteoprogenitor cells. It is not known whether osteoclasts may persist in a resting state. In some situations, cells that

are intermediate in position and appearance between osteoprogenitor cells and osteoblasts may be seen. These perhaps could be legitimately called preosteoblasts.

Chemical and histochemical studies *in vivo* and *in vitro* have greatly increased our understanding of the functional activities of the cells mentioned, as electron-microscope studies have greatly clarified our understanding of fine structure. Moreover, we have gone a long way toward correlating structure and function as a result of parallel morphologic and chemical studies.

The big questions which remain concern the factors governing the activity of the population, which control the mitotic activity of the osteoprogenitor cells and which direct their differentiation into active osteoblasts, chondroblasts, and osteoclasts. Many factors are known which are capable of stimulating changes in the appearance and activity of the population—trauma, hormones, nutritional disturbances, mechanical and electrical changes, and so on—but their precise modes of action remain to be discovered. Some of these factors seem to stimulate mitotic activity in progenitor cells; others seem to determine the course of differentiation; and still others may direct the functional activity of the differentiated cells.

In the normal skeleton, the bone-cell system varies greatly in its activity in different places and at different stages of development. Nevertheless, from the time of its inception in the embryo, the system remains throughout the life cycle of an organism either in an active or a resting state. However, bone-cell (and cartilage-cell) systems can arise spontaneously or, following experimental procedures, can be produced in connective tissues which do not normally ossify or chondrify. This is of great theoretic importance, for it implies that a connective-tissue cell population has received new instructions which supersede those they normally receive in development. This brings us into the realm of DNA control by the extracellular environment and the Jacob and Monod (ref. 211) theoretic systems.

This kind of change in cell behavior is called induction, and the factors which bring about the change are called inducing agents or, simply, inductors. As yet, we do not know what these inducing agents are, where they come from, or how they work. It is suspected that, in an inductive situation, one set of cells instructs the DNA of another set of cells—but it is by no means ruled out that some product of cell or matrix breakdown might produce induction, or even that the stimulus is a physical one.

There is one debate of special importance which concerns whether the cells are induced to change their way of life by a single "trigger" stimulus, or whether there is a series of continuing stimuli. Then, again, once a cell has been induced, does it stay induced indefinitely

throughout its own life and the life of its descendants? Or will it revert to its original state as soon as the inducing stimulus ceases? The extreme view is that all cells maintain their form and behavior only so long as they are being stimulated from outside to do so. The classic view, derived from experimental embryology, is that induction is permanent. It may well be that each cell type must be investigated on its own merits in these respects.

Finally, there was a lengthy argument about the relationship of mitosis to induction. On the one hand, it was suggested that only cells recently "born" are capable of responding to inductive agents; on the other hand, it was argued that certain cells might change their mode of life long after the mitosis which gave them birth. Of course, it is conceivable that a cell might receive instructions to become a bone cell, but not respond to those instructions for several weeks, behaving meanwhile like a perfectly ordinary-looking fibroblast; but is this what we usually think of as "induction"? In short, there is an urgent need to define what we mean by induction.

URIST: Thank you very much. Dr. Fremont-Smith has a few comments to make.

FREMONT-SMITH: I made a remark yesterday that cellular differentiation involved the inheritance of acquired characteristics and then I stopped at that point, and this needs to be elaborated.

I merely want to point out that the genes provide the potentials; the environment determines which potentials are going to be realized, and the environment is always present. When a cell is differentiated, it is differentiated as a result of environmental interaction with the gene potentials, and if the cell then gives rise to donor cells of the same specified type, this is, in fact, the inheritance of characters that the cells achieved through the environment. If enough were known about the environment and if it were appropriately modified, the manifestation would also be modified. There is no genic determination which does not involve a crucial aspect of the environment, and if that crucial aspect of the environment is modified, you no longer get the genic determination.

Lastly, I would give two examples. One is the loss of genes from the environment by radiation. This can lead to mutation, which is then inherited. This is an inheritance of a lost character, and the loss came about as a result of environmental influence. Transduction is the opposite—the achievement of new genes coming from a virus—and these new genes then give rise to a mutation, and this mutation breeds true to life; this, then, is the inheritance of a characteristic which came from the environment.

So, although people do not like me to say this, they usually do not deny the facts on which it is based. They wish I would use some other

words. I am not trying to support Lysenko's data, which I think were no good, but what I am saying is that the idea, the dogma, that we are now getting—that we have genetically determined characters—has to be modified to include the fact that there are no genetically determined characters which are not equally determined by the appropriate environment. I wish we had more time to discuss this, but there just is not enough time at this session.

SAXÉN: Dr. Urist said that I am not going to speak any more of the induction problem, but since we have repeatedly discussed the possibilities, and especially the limitations, of tissue-culture methods in studying bone development, it might be of interest to see a few illustrations.

We have recently tested the possibilities of analyzing the action of tetracyclines on calcification *in vitro*. Nevertheless, before any such quantitative determinations can be made, several factors need to be analyzed, and I will briefly mention some of them.

The first factor to exercise a major influence on the results is the age of the donor embryo. When two criteria were used, the length of the mineralized zone, and the total calcium of individual bones, the findings clearly indicated an optimal age of 17 days. Bones from embryos younger than this did, in fact, develop and calcify, but at a rate slower than those of the 17-day embryos, and when the rudiments were removed from 18- to 19-day embryos, the growth rate slowed down once more. These results thus indicate the importance of a homogeneous starting material in such experiments *in vitro*. The great influence exerted by age is clearly discernible in figure 144, which illustrates the incorporation of radiocalcium into bones of 16- and 17-day embryos, respectively.

In our different experiments, the tissue-culture medium had to be changed frequently (incorporation experiments, transfer to and from the tetracycline-containing medium, etc.). This led to the effect of such repeated changes being tested with a somewhat unexpected result. Seemingly, one change during the 10- to 12-day cultivation period did not affect the growth and calcification, but if the change was repeated once after two days, the calcium uptake was definitely diminished. The explanation of this phenomenon might be found in a certain micromilieu provided by the tissue cultivated for some time and removed by repeated washings. In practice, this means that unnecessary changes of the medium should be avoided and, when a change has to be made, a similar manipulation should be carried out for the control cultures.

The next thing to be checked was the "aging" of the medium if changes were avoided. An experiment was made, in which bone rudiments were cultivated in a medium for 14 days, measured, and dis-

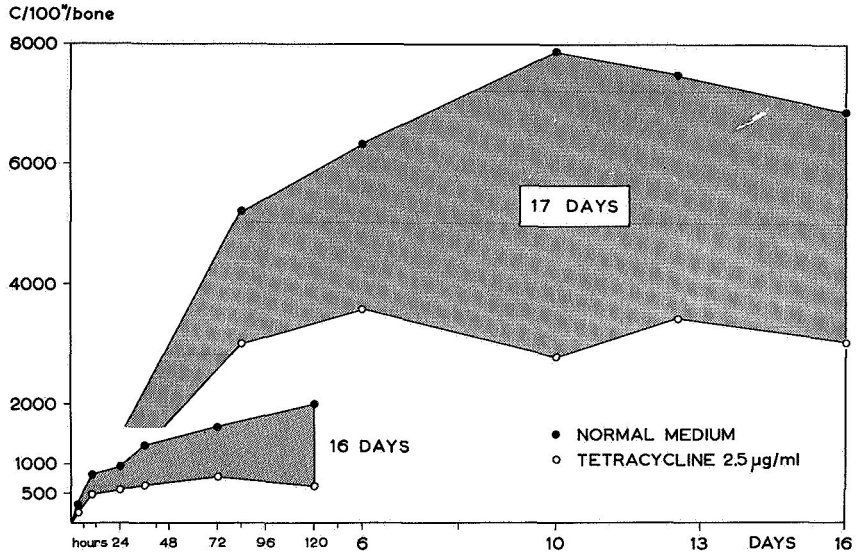

FIGURE 144. Incorporation of radiocalcium into bones of 16- and 17-day embryos.

carded. Subsequently, the same medium was reused for a similar culture, and the growth of the bones was again studied. The results clearly indicated that, at least during this 28-day period of total cultivation time, the same medium supported good growth, elongation, and calcification.

So much for the technical difficulties and limitations. If I may, I will very briefly go through some of the results, indicating what kinds of information we can and cannot expect from tissue-culture studies.

RAISZ: I think this is fascinating, and it seems astounding to me I wonder if you could tell us what the volume of medium was, relative to the amount of culture material.

SAXÉN: It was 1 to 100.

RAISZ: That is the usual tissue-culture ratio.

SAXÉN: Yes. Then, of course, we had to determine the total calcium and ionic calcium in the medium, and the changes are very slight.

One of the things for which our tissue-culture conditions seemed to be suitable was study of the incorporation of tetracycline into the bones. Frozen sections, made after different lapses of time, and studied in UV light showed a very rapid incorporation of the drug into the rudiments; even after some 30 minutes, clear fluorescence was visible. Later, this incorporation was determined by the employment of labeled tetracycline. Figure 145 shows the total incorporation

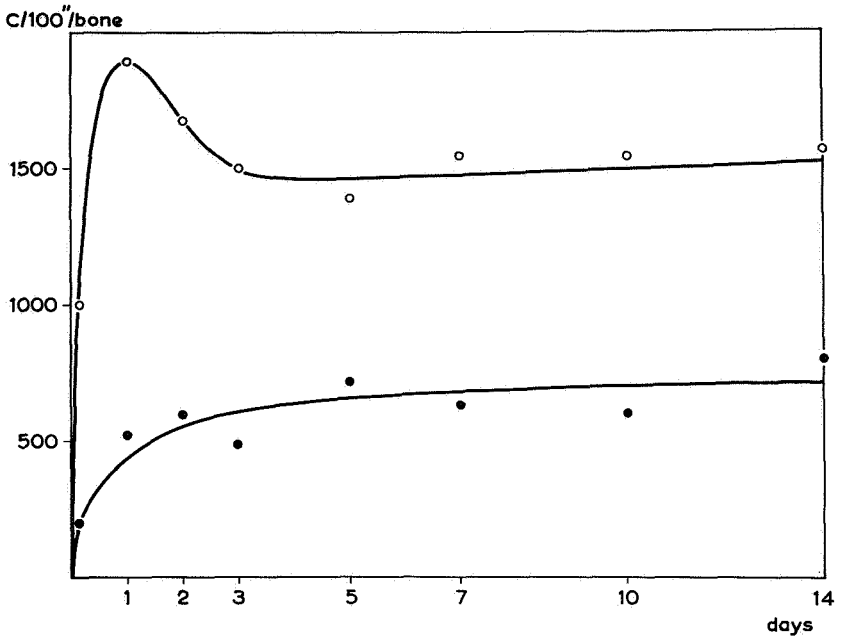

FIGURE 145. Incorporation of tetracycline into embryonic bone rudiments.

as a function of time. The upper line indicates the content of tetracycline in the bones immediately after treatment, and the lower line the content after 24 hours' subsequent cultivation in a cold medium. The results indicate that approximately four-fifths of the incorporated tetracycline leaks out rapidly, whereas one-fifth is retained in the bones. Figure 146 shows this leakage in more detail, and suggests that the portion of tetracycline retained in the bones after the first 24 hours will remain in the bones without any major changes.

BAUER: Which method did you use for measuring the amount of tetracycline?

SAXÉN: Tritiated tetracycline was counted in a liquid scintillation spectrometer after short-term treatment of the bones with hyamine.

Figure 147 illustrates the dependence of concentration on the incorporation of radiocalcium. It seems that a concentration of 0.1 μg/ml of tetracycline does not affect the uptake of calcium during this period of cultivation, whereas a definite diminution was noted at a concentration of 1.0 μg/ml and 10.0 μg/ml.

HOLTZER: After the tetracycline, do you think your system is still living? I was not quite sure I followed that.

SAXÉN: You mean whether the bones are living? Yes, they are.

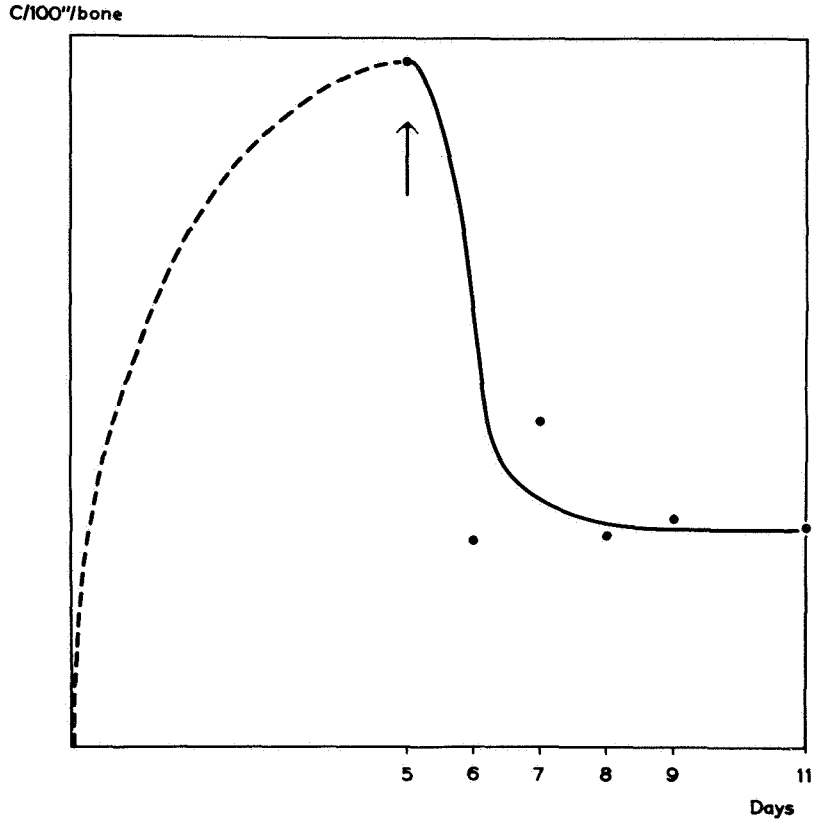

FIGURE 146. Incorporation of tetracycline into embryonic bone rudiments as a function of time.

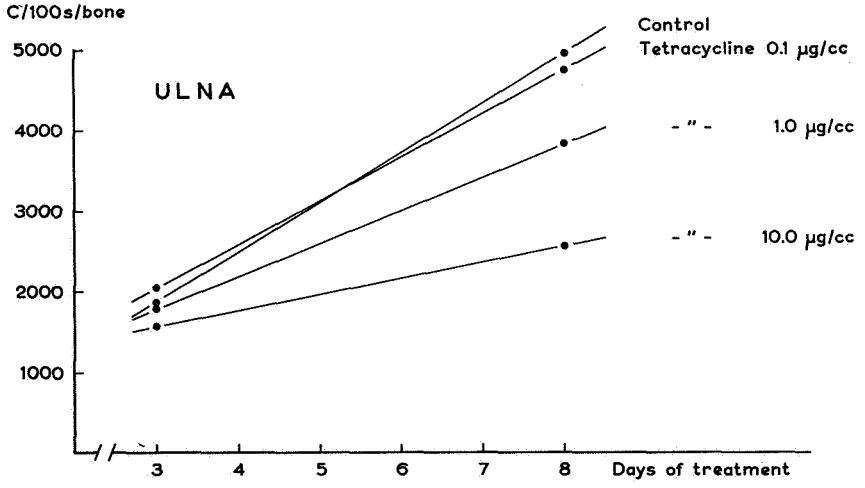

FIGURE 147. Dependence of tetracycline concentration on the incorporation of radiocalcium in embryonic ulna.

HOLTZER: And you can show this by putting them back in labeled calcium which they take up?

SAXÉN: Yes. I will come to that soon. The actual experiment which demonstrates the viability of the bones was performed by application of the thymidine leakage method. Here, the rudiments were labeled with ^{3}H-thymidine prior to tetracycline treatment, and later the amount of label in the culture medium was studied. No differences were noted between the control cultures and the cultures cultivated in the presence of tetracycline up to 100 μg/ml.

HOLTZER: Can you say something about the place in the tissue? Is this definitely an extracellular location which is not affecting cell viability on the part of tetracycline? It would be very nice if you could.

SAXÉN: At the moment, we do not know much about the mechanism, but we are investigating that in another system—a continuous cell line. With respect to the bone cells, I really believe that they are still alive after several days of tetracycline treatment. This is observable from experiments in which the bones have been cultivated in the presence of tetracycline for some days and then transferred to normal medium. Within a day or two, a recovery is achieved, and the bones incorporate radiocalcium at a normal rate. Not until 5 to 6 days of cultivation does the effect seem irreversible.

Even here we have good evidence that the cells survived. They are alive, but there is no recovery from the sixth day onward.

This summarizes my concluding remarks. I merely wanted to show one example of how organotypic culture methods can be used in studies of hard tissues, particularly bone.

URIST: Would you like to speculate on the site of action of the tetracycline in the cell?

SAXÉN: How it acts?

FREMONT-SMITH: How and where; both.

SAXÉN: Many people have speculated on that. One possibility, of course, is that it seems to interfere with the deposition of calcium into the crystals. At present, we are interested in knowing whether it interferes with collagen synthesis, and we are going to use proline incorporation and electron-microscopy studies to do that. As yet, I have no conclusive data. That is why I was very much interested, Dr. Talmage, in your similar study with salicylates—where you get an inhibition of the collagen.

BAUER: In your article in *Science* (ref. 214), you made the point that you found the effect on collagen synthesis by tetracycline concentrations approximately the same as those encountered after tetracyclines have been administered for therapeutic reasons.

SAXÉN: The therapeutic concentration during a normal treatment is on the order of 4 to 5 μg/ml.

BAUER: Dr. Urist has reviewed evidence that tooth development has been found to be impaired in infants from mothers who have received tetracycline therapy during pregnancy.

URIST: We were very interested in the subject, because the mothers that had been treated with tetracycline during pregnancy for lung infection, and infants treated for various infections, presented a very disturbing problem. When the teeth of the child erupt, they are bright yellow and then turn brown; as you can imagine, it is a very disturbing sight to the mother. We performed experiments on rabbits and concluded that the tetracycline was bound to the crystal surface of apatite. After exposure of the teeth to sunlight, some tetracyclines are unstable, degraded, and turn from yellow to brown. Chlortetracycline is more stable than tetracycline phosphate (ref. 215).

HOLTZER: Why is it used in the clinic?

NICHOLS: It is an antibiotic.

BAUER: In tissue cultures, one usually studies effects of agents in very much higher concentrations than those normally encountered in real life conditions. This is why it is so highly interesting to find that effects of tetracyclines on mineralized tissues apparently occur *in vitro* and *in vivo* at approximately the same concentrations of this agent.

SAXÉN: If we can make any conclusions of the action *in vivo,* the concentration is of this very same order.

PECK: One of the problems associated with clinical use of tetracycline is that outdated or old tetracycline undergoes chemical changes which render it toxic to humans, particularly with respect to renal problems. I wonder if you have any evidence that the tetracyclines are not undergoing these changes in your culture medium.

SAXÉN: It must be a very rapid change, because we get the effect in 2 days with a fresh, new, and purified tetracycline preparation.

PECK: I do not know how it would behave under different circumstances. I do not think there is any evidence one way or the other.

NICHOLS: Dr. Holtzer, your question is one which you should never ask a clinician. There are many people who think these drugs are used far too indiscriminately in the practice of medicine. Tetracyclines are very popular because they have a broad spectrum of activity against a wide variety of organisms.

SAXÉN: It seems to be very popular for treatment of chronic urinary-tract infections during pregnancy, which I have been told are rather tricky.

NICHOLS: There are complications with the use of these drugs. While many people tolerate them quite well for long periods, secondary infections by nonsensitive organisms often develop during their use.

RAISZ: Dr. Saxén's very beautiful studies have brought up two points. One is the problem of dose, and I would like to rise to the challenge that Dr. Bauer has brought up on this score. In tissue culture, exogenous drugs can have effects in doses similar to those which act *in vivo,* unless there are specific carrier or conversion systems for the drug. Therefore, I do not think that we can say that tissue-culture systems always respond to concentrations higher than those of the blood. Dr. Peck has been able to get responses with ascorbic acid at concentrations which are close to those found in cells. I think many other tissue systems have been refined in tissue culture to the point where they show responses to a variety of agents at concentrations near those found *in vivo.* In this regard, I should like to show some data which indicate that parathyroid hormone in normal rat blood apparently affects the radiocalcium release from embryonic bone in tissue culture (table XXXIV).

These were made on heat-inactivated sera from normal rats and thyroparathyroidectomized rats. Calcium release measured in a medium containing normal rat serum is relatively high, and this value is lowered by the addition of serum from a guinea pig immunized with purified bovine parathyroid hormone. If one uses serum from thyroparathyroidectomized rats, there is a much smaller release of calcium, probably largely due to physical chemical exchange of the surface radioactivity with the medium, and there is no inhibition of this by antibody. A dose of 1 μg/ml of purified bovine parathyroid hormone added to serum from thyroparathyroidectomized rats caused a response

TABLE XXXIV

EVIDENCE FOR DETECTION OF PARATHYROID HORMONE IN NORMAL RAT SERUM BY BIOASSAY

Added serum (5 percent)	^{45}Ca release, cpm/0.1 ml of medium		
	Normal serum	TPTX serum	TPTX serum and PTH
Normal guinea-pig serum	2190 ± 310	[a] 1010 ± 150	2110 ± 150
Anti-PTH guinea-pig serum	[a] 1020 ± 150	[a] 1040 ± 170	[a] 1000 ± 20

NOTE.—Values are means ± S.E. of 4 bones.

[a] Significantly different from values in normal rat serum or TPTX serum plus PTH, $p < 0.02$. Data are for ^{45}Ca release from bone cultures in serum from normal or thyroparathyroidectomized (TPTX) rats. Effect on ^{45}Ca release of normal serum, or when bovine parathyroid hormone (PTH, 1 μg/ml) is added to serum from TPTX rats, can be abolished by adding serum from a guinea pig immunized with bovine PTH.

similar to that obtained in normal serum and this was also inhibited by antibody.

I believe that this type of data indicates that if we can overcome the problems of species difference and some of the difficulties in maintaining tissue cultures, we should expect to achieve responses to normal blood levels of hormones and to the usual *in vitro* doses of drugs. In other words, we should not accept the requirement of a high concentration *in vitro*, but should work toward showing effects with lower concentrations. One other point that I would like to make is that Dr. Saxén's findings are contrary to the usual dogma of tissue culture, which is that the medium must be changed frequently. I would like to hear his comment on this, because this dogma has been religiously adhered to and, of course, we could save a lot of work if Dr. Saxén would promulgate this new doctrine.

SAXÉN: It is very difficult to comment on that, because it is just a finding. I was surprised myself, and I have repeated the experiment. It may be that the metabolism of the bone is low—I do not know. Another thing which I tried to show is that in certain experiments the medium should not be changed, because a different effect is obtained.

NICHOLS: Dr. Saxén, one wonders immediately whether there is something accumulating in the incubation medium which is, so to speak, "good" for the bone. Have you done the critical experiment of taking the medium off the culture and then replacing it again? One wonders whether the actual maneuver of removal is important or whether it is the medium itself?

SAXÉN: I have not done this, yet I feel that it is the maneuver because in some instances we have to be very careful when we measure them daily. If the tissues are just shaken, decreased growth results.

HOLTZER: May I suggest that really the problem you are alluding to, at least as I can understand it, is that changing the medium promotes mitosis. Does a change in medium promote anything else? I am not aware of it. It might very well, but I know that when we have had occasion to measure, mitosis has always occurred. My bet would be that whatever the constant situation is after 6 or 7 days (and you say even changing the medium essentially limits it), mitosis is discouraged, and, conversely, in an obligatory way, the uptake of calcium is promoted, and you have an inverse ratio, whatever the requirements are for reproduction. This is the negative aspect in tissue culture.

SAXÉN: I do not want to go back to the environment-induction problem, but as you know, embryologists have repeatedly stressed the microenvironment, which cells and tissues create as they grow in tissue culture, so it may well be that you just wash it out.

NICHOLS: Yes, indeed. The reason I was intrigued was that I have found that the packing of the cells in bone-cell suspensions seems to be

a very critical factor. In my system, for instance, the oxygen uptake of the cell population per milligram of DNA increases as the concentration of cells in the medium increases, which is absolutely upside down from what one would expect if diffusion were a limiting factor.

BUDY: In defense of *in vitro* systems, I would like to point out that André (ref. 216) did a gross distribution study of tritiated tetracycline, and the distribution within bone certainly paralleled what you have shown so beautifully in your tissue-culture system.

SAXÉN: I may say that this is not the only report.

BUDY: There were several others, but André's thesis was——

SAXÉN: He was the first; others have made use of that typical fluorescence.

BUDY: There is a very close relationship between André's results and what you have shown. I am speaking in reference to gross autoradiography.

SAXÉN: I guess this may take care of one-third of the tetracycline. Where the rest of it is I do not know. We do know that dying cells do take up tetracycline, and that peak at the very beginning of any tissue culture may very well mean that there are dying cells taking up tetracycline; this fits with our observations of the thymidine leakage. There is a similar peak in the thymidine concentration of the medium at this time.

URIST: Dr. Arnaud, have you some material you would like to present?

ARNAUD: Yes, I think so. I should like to expand some on the comments I have made during the discussion and offer support for them with recently obtained data. First, the matter of using parathyroid extract in *in vitro* systems. When fractionating crude parathyroid extracts on Sephadex G–100 columns, as in the routine purification of parathyroid hormone, one obtains a series of protein peaks which have neither calcium mobilizing nor phosphaturic activities in the parathyroidectomized rat (ref. 217). Hawker and Glass have purified two of these and have characterized them chemically. They bear no relationship to the classic parathyroid hormone in this regard either. Nevertheless, they have interesting properties in *in vitro* systems. Dr. Tenenhouse has shown that one of them, peak 3, stimulates glycolysis when added *in vitro* to ascites tumor cells, an effect reminiscent of that which has been reported when parathyroid extract has been added *in vitro* to ascites tumor cells, and also reminiscent of that which has been reported when parathyroid extract has been added *in vitro* to incubated bone chips. The other polypeptide, peak 2, has been studied extensively in mitochondrial systems by Rasmussen and Ogata (ref. 218). Superficially, its effects resemble those of parathyroid hormone in that it stimulates mitochondrial phosphate accumulation.

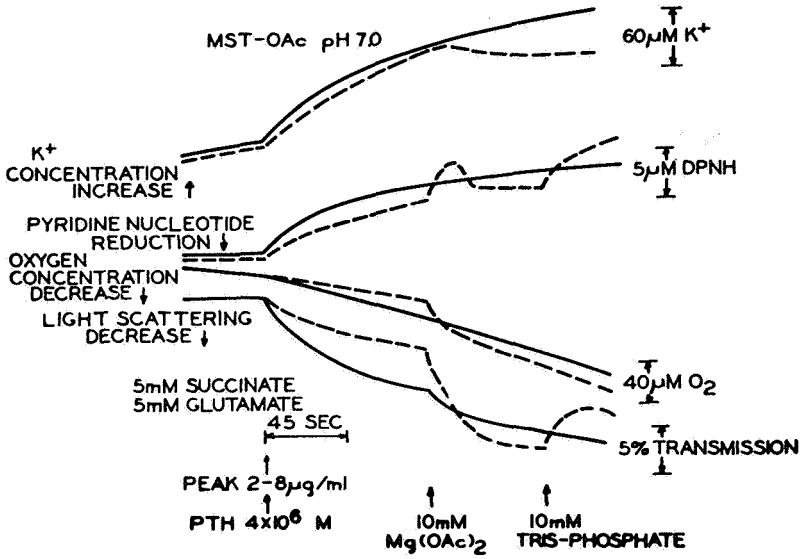

FIGURE 148. Responses of isolated rat liver mitochondria to successive additions of 10 millimoles $Mg(OAc)_2$ and 10 millimoles *Tris*-phosphate in the presence of 8 μg/ml of peptide, peak 2 (———) compared with the responses seen in the presence of 4×10^{-6} *M* parathyroid hormone (- - - - -) in a mannitol-sucrose-*Tris*-acetate medium. [From ref. 218; reprinted by permission of the publisher.]

However, using more discriminating methods, it is easy to show that the effects of these polypeptides on mitochondrial metabolism are quite different. Figure 148 shows the effects on the oxidation of pyridine nucleotide, oxygen consumption, and light scattering (mitochondrial swelling) of rat-liver mitochondria incubated in a mannitol-sucrose-acetate medium at pH 7. Parathyroid hormone causes only small changes in these parameters until either magnesium or potassium is added. At this time, there is rapid pyridine nucleotide oxidation, increase in oxygen consumption, and mitochondrial swelling. Partial reversal of swelling is effected by the addition of phosphate. In contrast, the addition of peak 2 is followed immediately by irreversible changes in all indices. Neither magnesium nor potassium is required for these effects.

I think that we can assume that both of these peptides (peaks 2 and 3) are present in parathyroid extracts which are commercially available at this time. The use of the extract in studies *in vivo* is probably safer than studies *in vitro*, but I personally use great caution when interpreting any work done with material other than the purified preparation.

Now we will shift to another topic. To employ physiologic techniques in some of our biochemical studies, we found it necessary to

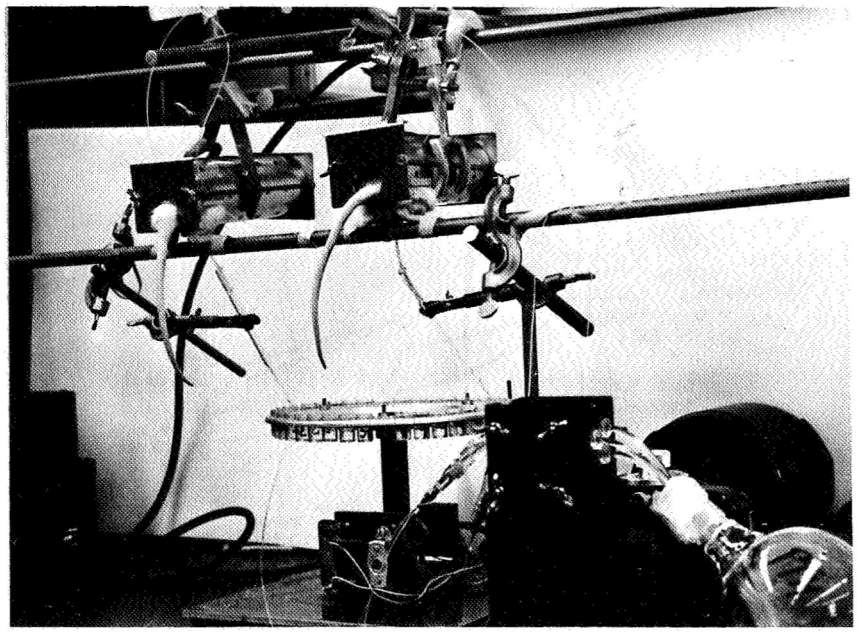

FIGURE 149. Two thyroparathyroidectomized rats set up for the long-term perfusion of parathyroid hormone.

develop a relatively precise technique for the study of the effect of hormones *in vivo* (ref. 54). Figure 149 is a picture of two rats that, believe it or not, are relatively comfortable. They are either parathyroidectomized or thyroparathyroidectomized and can be maintained for up to 4 days by perfusing them at a constant rate with an electrolyte solution which contains calcium, sodium, potassium, magnesium, and glucose via a polyethylene catheter inserted into the external jugular vein. Another polyethylene tube is placed in the urinary bladder to collect urine. A fraction collector is used and urine samples of any time interval can be obtained so that a semicontinuous monitoring of urinary electrolyte composition is easily accomplished. Also, blood can be obtained for analysis at judicious times during an experiment by slashing the animal's foot with a sharp razor blade. Animals are perfused at rates of 3 to 4 milliliters per hour, and there is no significant water retention. These techniques are modifications of the procedures described by Cotlove (ref. 219) and by Pechet.[1]

The work I would like to show you now was done with Dr. Anast during the year he spent in our laboratory on leave from the Department of Pediatrics at the University of Missouri School of Medicine

[1] Pechet, M. Personal communication.

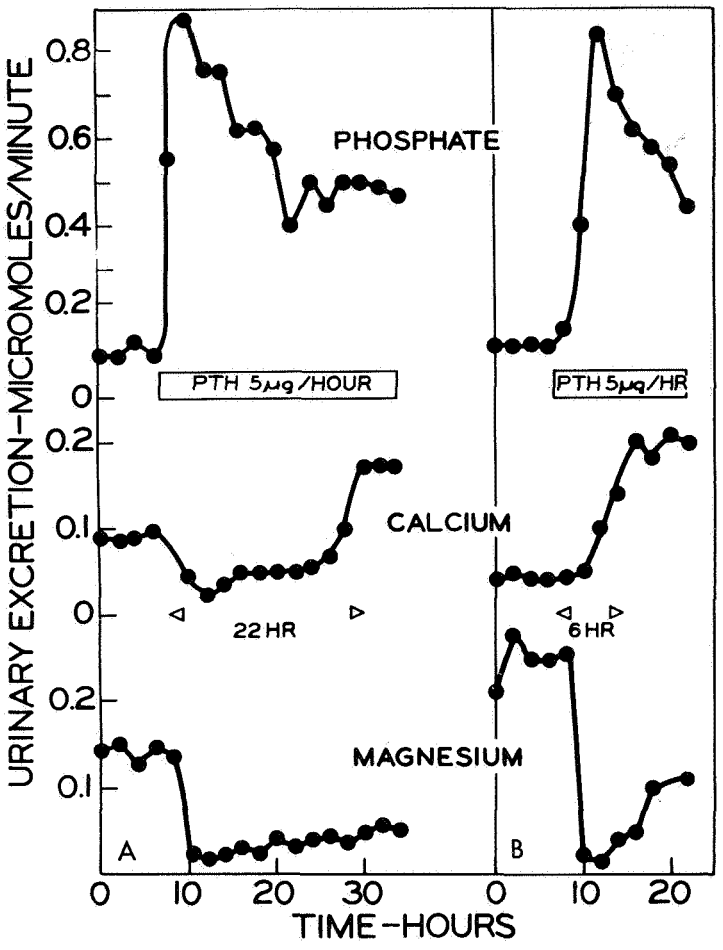

FIGURE 150. The rate of excretion (μmoles/min) of phosphate, calcium, and magnesium in the urine of parathyroidectomized (*a*) and thyroparathyroidectomized rats (*b*) before and during the constant infusion of parathyroid hormone at a rate of 5 μg/hr. The values represent the means obtained from four rats in each set. [From Anast et al. (ref. 220); reprinted by permission of the publisher.]

(ref. 220). It was designed to demonstrate the importance of thyrocalcitonin in the responses of the rat to an excess of administered parathyroid hormone and employed the rat perfusion system I have just described.

Figure 150 compares the responses of the parathyroidectomized (PTX) rat, on the left, with the thyroparathyroidectomized (TPTX) rat, on the right, to a constant infusion of parathyroid hormone (5 μg/hr). There is a rapid and sustained phosphaturia in both animals, but it is

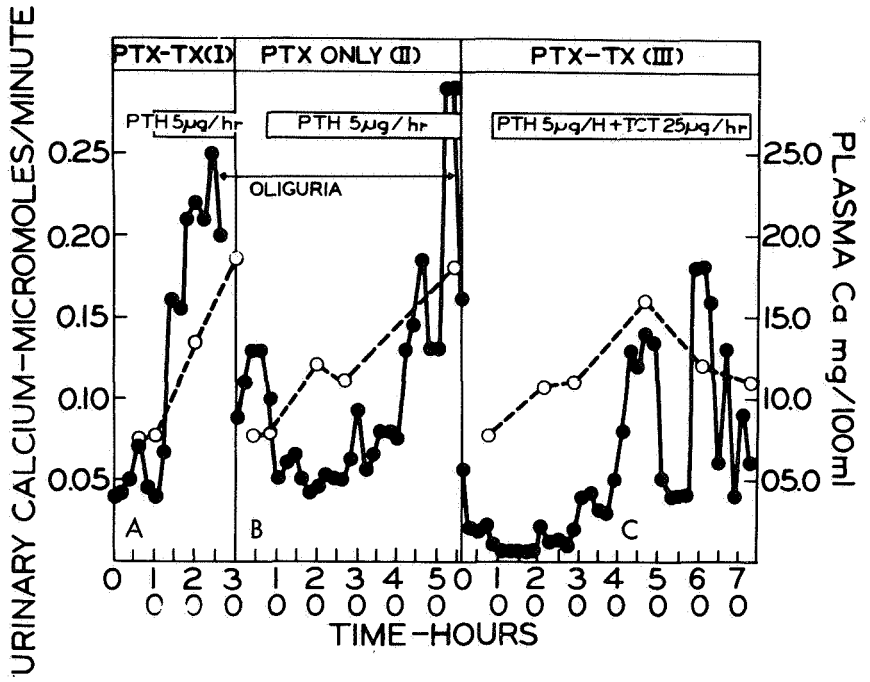

FIGURE 151. Plasma calcium concentration (○- - - -○) and rate of urinary calcium excretion (●——●) before and during the infusion of purified parathyroid hormone in (*a*) a thyroparathyroidectomized rat; (*b*) a parathyroidectomized rat with an intact thyroid gland; and (*c*) a thyroparathyroidectomized rat receiving a simultaneous infusion of thyrocalcitonin (25 μg/hr). The thin arrow with *oliguria* written under it indicates that oliguria developed after 16 hours of parathyroid hormone infusion in rat A and after 48 hours in rat B. No oliguria was seen in rat C. The values represent the means obtained from four rats in each set. [From Anast et al. (ref. 220); reprinted by permission of the publisher.]

clear that their responses differ with respect to calcium excretion. The animal without thyroid-parathyroid glands develops hypercalciuria within 6 hours and dies within 14 to 16 hours of hypercalcemia and nephrocalcinosis, whereas hypercalciuria is markedly delayed in the animal with a thyroid gland and nephrocalcinosis is rarely observed. It is interesting, with regard to our previous discussion concerning the rapid action of parathyroid hormone, that a detectable increase in the plasma calcium concentration can be observed within one-half hour in the TPTX rat, but not until 2 to 3 hours in the PTX rat. We think that the differences between the responses of these animals is the presence of circulating thyrocalcitonin in one and its absence in the other.

Studies of the influence of the concomitant perfusion of purified thyrocalcitonin on the pattern of response of the TPTX rat to parathyroid hormone support this notion. Figure 151 illustrates this,

and shows clearly the importance of the presence of the thyroid gland when one attempts to interpret the physiologic or pharmacologic effects of parathyroid hormone administration. Either the presence of the thyroid gland (fig. 151(*b*)) or the perfusion of thyrocalcitonin (fig. 151(*c*)) dramatically protects against the hypercalcemic and toxic effects of parathyroid hormone observed in the animal without thyroid-parathyroid glands or thyrocalcitonin (fig. 151(*a*)).

I should like to point out, in my last few remarks, a finding which we have only recently observed (fig. 152). Using the rat perfusion system, we have been able to show that the infusion of thyrocalcitonin into TPTX rats either alone (fig. 152(*b*)) or with a maintenance dose of 1 μg/hr of parathyroid hormone (fig. 152(*a*)) induces transient phosphate and sodium excretion, and a sustained decrease in the rate of calcium and magnesium excretion. The important question is whether this is a

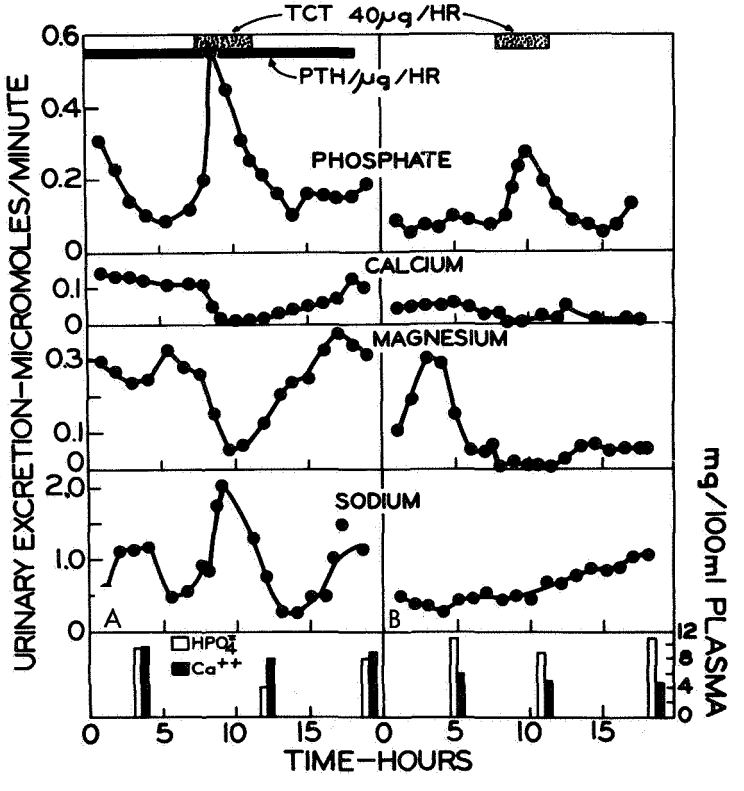

FIGURE 152. The effect of thyrocalcitonin (TCT) infusion upon urinary and plasma electrolytes in thyroparathyroidectomized rats (*b*) and similar animals maintained on a constant infusion of parathyroid hormone (*a*). The values represent the means obtained from four rats in each set.

direct effect of thyrocalcitonin or is related to changes in the local ionic environment of the kidney tubule. This is currently under study. Of great interest is the fact that the total phosphate which is excreted during thyrocalcitonin administration corresponds closely to the calculated decrease in the phosphate content of the extracellular fluids. This immediately raises the question as to whether the hypophosphatemic effect of thyrocalcitonin, under physiologic conditions, is due exclusively to its influence on bone.

URIST: Thank you very much. Dr. Copp, have you any comments?

COPP: I believe Munson (ref. 221) also observed an effect on phosphate in the nephrectomized animal.

ARNAUD: One thing, of course, that we do not know very much about is soft-tissue phosphate. As a matter of fact, we do not know a great deal about soft-tissue calcium either, and this is a matter which requires considerable study, as it is extremely difficult to measure soft-tissue calcium accurately.

COPP: Despite blood changes, Kenny and Heiskell (ref. 222) reported no significant changes in intracellular calcium and phosphate following thyrocalcitonin administration to rats.

ARNAUD: It must be remembered that every tissue is perfused by large quantities of extracellular fluid. If there is a decrease in the extracellular fluid calcium, one would also expect a decrease in the cellular calcium, unless careful measurements of the extracellular fluid space of the tissue and its calcium content are taken into consideration.

COPP: There is no indication that hypocalcemia is associated with any significant reduction in intracellular calcium, although extracellular calcium is reduced.

ARNAUD: Yes; but the tissue calcium is measured along with the extracellular fluid calcium. None of the studies done so far has accounted for the extracellular space.

COPP: I assume that it drops with the fall in plasma calcium concentration. It must.

ARNAUD: I am only suggesting that, on the basis of precedent (actions of other hormones), tissues other than bone might be responsive to thyrocalcitonin.

COPP: I think that this is a very important aspect of phosphate metabolism. There must be rapid movement of phosphate in and out of cells with changes in metabolic activity, since phosphate is required for the high-energy phosphate bonds involved in energy transfer.

FREMONT-SMITH: A factor that seems to have been regularly neglected is that probably the tissue that has the most constant calcium content in the body is cerebrospinal fluid. This does not vary, even with marked variations in plasma calcium concentration, whether the plasma

calcium is raised and maintained in the dog for several days at 12 to 14 mg/100 ml, or whether it is decreased by lowering the plasma calcium to the concentration you get after the thyroid has been removed. But the only point at which the spinal-fluid calcium begins to decrease is the point at which the plasma calcium falls below 4.5 or 4.0 mg/100 ml, which is the concentration of ionized calcium or that of the spinal-fluid calcium. In the very few instances which I have seen in the literature, the spinal-fluid calcium concentration then drops down to about 3, right with the plasma calcium.

These are, of course, extremely low concentrations; a comparable result is rarely obtained. Everybody ignores this because they insist that spinal fluid has nothing to do with calcium anyway—"It is a secretion; therefore, let us forget about it." But it is the most constant tissue fluid for calcium in the body, I believe.

ARNAUD: I think the fact that these agents do not affect the membranes which are responsible for the regulation of the spinal-fluid calcium is terribly important in terms of brain homeostasis.

NICHOLS: There are some experimental data on this subject from a couple of laboratories, notably Katzman's. These suggest that spinal-fluid calcium concentration is closely controlled independently of the concentration in the plasma. It looks as if there may be a transport system somewhere in the brain, presumably with its own "calciostat," if one may use that word, which controls cerebrospinal-fluid calcium concentration by transfer of calcium from blood to the cerebrospinal fluid (ref. 223).

FREMONT-SMITH: Does anybody know what happens to the spinal-fluid calcium when a considerable amount of citrate is injected into the blood? I have always thought that this was one way in which it could be determined whether changes in the spinal-fluid calcium occur.

COPP: I can give some observations made by Dr. Shim [2] in our laboratory on the effects of EDTA. When the noncomplexed plasma calcium concentration is reduced as low as 2 mg/100 ml by rapid EDTA infusion, there is no tetany.

FREMONT-SMITH: What happens to the spinal fluid?

COPP: Spinal-fluid changes lag about 3 hours behind plasma.

FREMONT-SMITH: But it does come down?

COPP: It does come down.

FREMONT-SMITH: This lag, by the way, is characteristic of almost everything in spinal fluid.

---

[2] S. S. Shim, Department of Physiology, University of British Columbia, Vancouver, Canada. Personal communication.

COPP: Yes; there certainly is such a lag. However, if you lower the spinal-fluid calcium but keep plasma calcium normal, the animal has muscle spasms resembling severe tetany.

FREMONT-SMITH: How do you lower the spinal-fluid plasma?

COPP: By injecting EDTA into the spinal canal. This would suggest that the tetany may be a central rather than a peripheral phenomenon.

URIST: Dr. Arnaud, is total soft-tissue calcium, total extracellular fluid calcium plus intracellular fluid calcium, difficult to measure accurately?

ARNAUD: No. I think there are problems with respect to interfering substances such as phosphate; so much so that when we came to measure calcium in mitochondria, we went to activation analysis and found that even this was somewhat difficult in terms of reproducibility.

URIST: Dr. Heaney, have you made measurements of soft-tissue calcium?

HEANEY: No.

COPP: We measured it with EDTA (ref. 224).

URIST: Phosphate does not interfere with the EDTA method unless there is hyperphosphatemia or large amounts of phosphorus in the tissue.

COPP: We find, with hypocalcemia, you have a slight fall which I have attributed to the fall in extracellular fluid calcium. With calcium perfusion, you have a slight increase, but I think this is accounted for almost entirely by extracellular fluid calcium.

ARNAUD: The point is that it can be done. It is just very difficult to do.

HEANEY: I will second that. It is very hard to do and it is very tedious. There is a great deal more calcium in soft tissue than can possibly be accounted for by the extracellular fluid content. It is not in the cell sap.

COPP: Especially in tissues with a high level of sialic acid or mucopolysaccharides. For example, there is a high calcium concentration in thyroid and salivary glands.

HEANEY: Skin has 150 to 250 milligrams of calcium per kilogram.

HOWELL: Hypertrophic cell cartilage contains twentyfold to thirtyfold per unit of wet-weight calcium measured grossly. We obtained some of our samples from muscle insertion sites, and that is probably fairly close to an extracellular fluid. It has 1 gm/100 ml protein and the calcium measured in that was about 7 mg/100 ml.

PECK: It has been postulated that there is actually an ion cloud which is very rich in calcium that surrounds many cell types, particularly the muscle cells, and if the concentration of calcium in the fluid which

surrounds all cells is measured, it increases dramatically, the closer you get to the cell membrane.

ROWLAND: What mechanism can control this?

PECK: Well, it possibly has to do with the actual composition of the cell membrane itself. There is very little information about this.

URIST: Dr. Peck, have you some material you would like to present?

PECK: Yes. We have been discussing the problem of membranes, and the concept that hormones, inducers, and stimulators act by modifying the function membranes is not a new one. We have been interested in the possibility that hormones can modify the lipid composition of cell membranes, in particular the membranes of our isolated bone cells, and it is only recently that the techniques for isolating and separating lipids have become sophisticated enough to be applied to relatively small amounts of tissue.

Much of the work in designing the techniques for these separations was done by Marinetti in 1962 (ref. 225). I have done these studies in collaboration with Dr. Thomas Dirksen in the Department of Biochemistry (ref. 226). The main question, or the only question, that we have answered to date is, Can the cells synthesize lipid, and if so, what kind of lipid can they synthesize?

Figure 153 shows the time curve of incorporation of ^{14}C-glycerol into the lipids of cells maintained in culture on a flat surface (ref. 227). Most of the lipid that is formed appears in the cell layer. A very small percentage appears in the supernatant medium that overlies the cell layer.

The interesting thing is that the large percentage of incorporated glycerol appears in phospholipid which, as you all know, constitutes the majority of lipid in cell membranes. A large proportion of the radioactivity is in lecithin or choline phosphoglyceride.

There is an appreciable incorporation of glycerol into neutral lipid and a small but significant incorporation into phosphatidyl ethanolamine and other phospholipids.

I think the only implication is that we have a method for studying the possible mechanism of action of hormones at the level of the cell membranes. It is possible, for example, that a hormone can alter the transport processes of cell membranes by actually altering those factors that modify the lipid composition of the cell membrane. We have recently done some studies on the addition of parathyroid extract—and I must emphasize, in view of Dr. Arnaud's comments, that this is the crudest material—on the ability of these cells to synthesize lipid. We have found some evidence to suggest stimulation of incorporation of glycerol into phospholipid moieties, particularly lecithin and phosphoethanolamine. We have also tried one experiment with a more

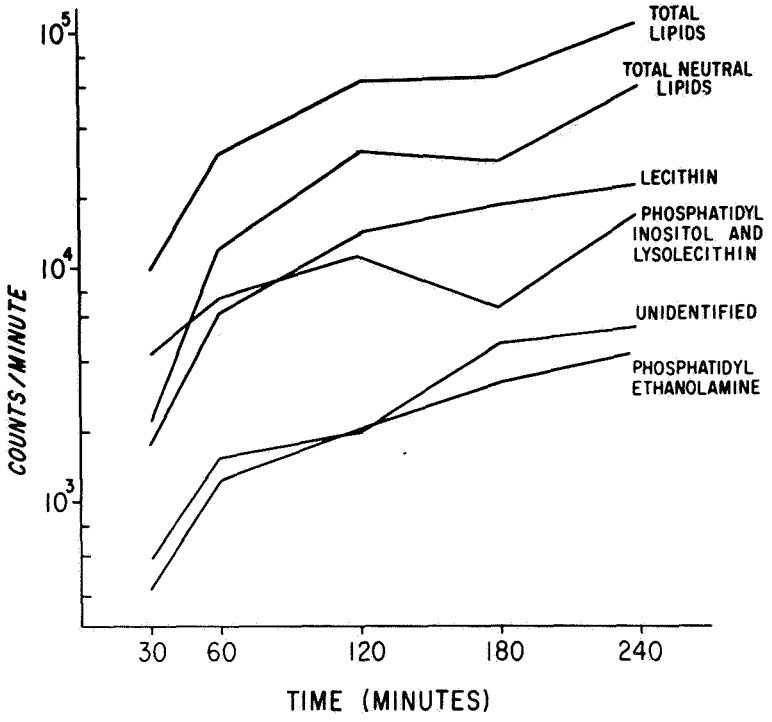

FIGURE 153. Time curve of incorporation of 1,3-^{14}C-glycerol into the phospholipids of rat calvaria. After incubation, lipids were extracted into chloroform-methanol and separated by chromatography on silicic acid-impregnated paper. Papers were cut into horizontal strips corresponding to the position of standards chromatographed simultaneously, and each strip was counted in a liquid scintillation spectrometer. [From ref. 227; reprinted by permission of the publisher.]

purified, but not completely purified, preparation, and the data suggest again a possible stimulation of this incorporation. If subsequent studies confirm this, we will direct our attention to isolating various cell fractions to see whether these changes are reflected, for example, in the lipid composition of the mitochondria, the plasma membrane, and so forth. I just think it is worth mentioning because of the possibility—well, the probability—that much of our attention about the mechanism of hormone action is going to be directed in the near future toward the function of various membranes—cellular and intracellular.

ARNAUD: I think this is going to be a very profitable approach. I hope that you will couple some studies that you are going to be doing on lipids with ion flux studies, because I think this is most important. The correlation is going to be terribly important, especially the time correlation.

PECK: The point that you make is a good one. Interpretation of these kinds of data must be done with extreme caution. In this kind of system, there is no telling what comes first, the chicken or the egg.

NICHOLS: I think you are involved, too, in the question of whether you may be dealing simply with transfer of precursors.

HOLTZER: In this context, I should like to mention some experiments that Dr. Nameroff and I are engaged in. It is a demonstration of what you can do with tissue culture, worrying about cell membranes.

Yesterday I talked of how liberated cartilage cells, if grown in a dense culture, would form a layer of cartilage within 5 or 6 days (ref. 82). Now, if freshly liberated cartilage cells are added to these cultures, the added cells are not induced to reenter the mitotic cycle as they would be if they were plated out on glass. Chondrocytes plated on glass rapidly flatten and their cell surface increases sixfold in a matter of hours. These are the cells that are induced to make DNA. However, when these cells are added to a sheet of preformed cartilage, they do not flatten and they are not induced to make DNA. They remain round and make chondroitin sulfate. Our current interpretation is that the permeability of the rounded chondrocyte is quite different from that of a stretched-out one, and that this type of difference leads to profound differences in the activities of the respective cells.

FREMONT-SMITH: You are showing how important the environment is in the specialized functions of the cell. I had to throw my concept in on that.

NICHOLS: You wanted to add a shape to that.

FREMONT-SMITH: The environment determines the shape in this case.

NICHOLS: May I talk about what may be an example of a neutral induction system?

URIST: Please do.

NICHOLS: Yesterday I started to tell you something about multiple myeloma, which I would like to suggest may be an example of an induction system at work.

Multiple myeloma, for those of you who are not clinicians, is a disease which affects bones. Bone destruction is seen which is thought to be the result of an invasion of an abnormal form of a connective tissue cell—the plasma cell. There are various bone lesions ranging from "osteoporosis," which is just osteopenia by X-ray, to multiple punched-out lesions. The latter contain masses of abnormal plasma cells and are called plasmacytomas. The bone cells themselves appear histologically normal.

The data I would like to show are of the same sort as those I showed before and are taken from a recent publication (ref. 228). Included

are data concerning bone, the myelomatous marrow that has been washed out of the bone, and plasmacytomas as examples of pure cultures of abnormal plasma cells. The reason for studying marrow and plasmacytomas as well as bone cells was that the changes we hoped to find in the bone might be simply due to contaminating myeloma cells—a fact which might go unrecognized if we did not learn about the plasma cells.

Figure 154 summarizes the data on bone-cell metabolism. The shaded areas indicate the range covered by two standard deviations above and below the mean normal value as before. Individual patients are shown by number so that the combination of metabolic values for any individual may be readily identified.

Clearly, there are patients in this group whose bone cell O_2 uptake and lactate production are abnormally active, but the data are too scattered to allow a characteristics pattern to be identified. The data concerning proline metabolism are those I would like to emphasize. Seven of the 10 showed slight-to-marked increase in retention of proline label in the bone cells, but only two showed any evidence of the concomitant increase in labeling of the collagen of the samples, and of these only one was clearly high. While increased labeling from glucose was also present, the dichotomy between the changes in cellular and collagen labeling was not preeminent.

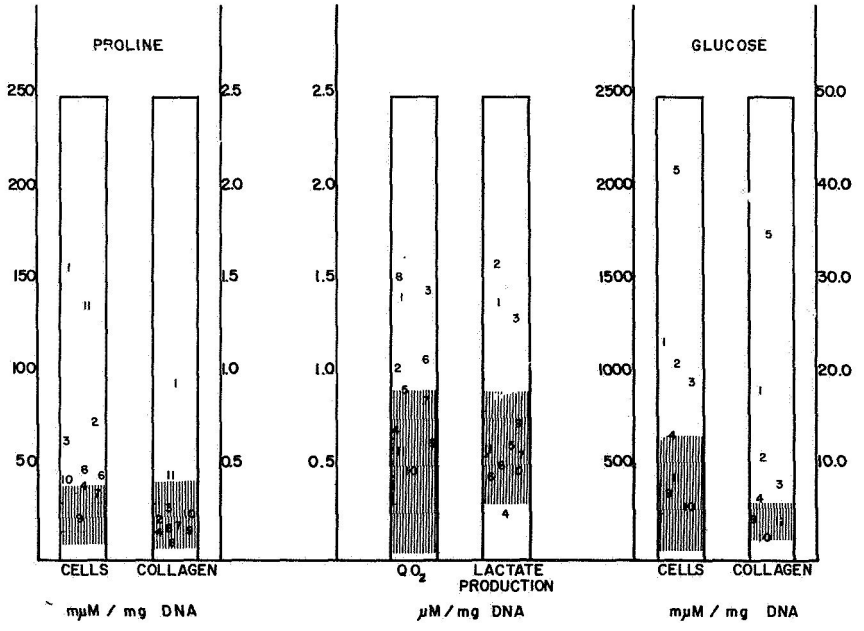

FIGURE 154. Bone metabolism in multiple myeloma.

These findings raised two questions: What was the excess proline label in? What was the contribution of marrow contaminants to these changes? The answer is largely in a cell fraction insoluble in 3 percent trichloroacetic acid, suggesting incorporation into a macromolecule—perhaps protein—but this material remains to be identified.

The answer to the second question is in part supplied by the data in figure 155. Most of these data fall within the range of values obtained in the few normal samples of marrow which we have examined. Of particular importance was the fact that the proline incorporation into myelomatous marrow cells was not different from controls. Since clear correlations between changes in marrow and bone cells could not be found, it has been tentatively concluded that marrow probably contributed little to the apparent changes which we found in bone-cell metabolism.

One other observation from this study might be of interest, although it is negative. The possibility that the excess proline retained in the cells might be in a protein suggested to us the possibility that collagenase is being made in excess in these cells and might be responsible for the excess bone breakdown. Collagenase activity was, therefore, measured. The data obtained are shown in figure 156. Again, the normal range is shown by the shaded area. Collagenase activity was normal

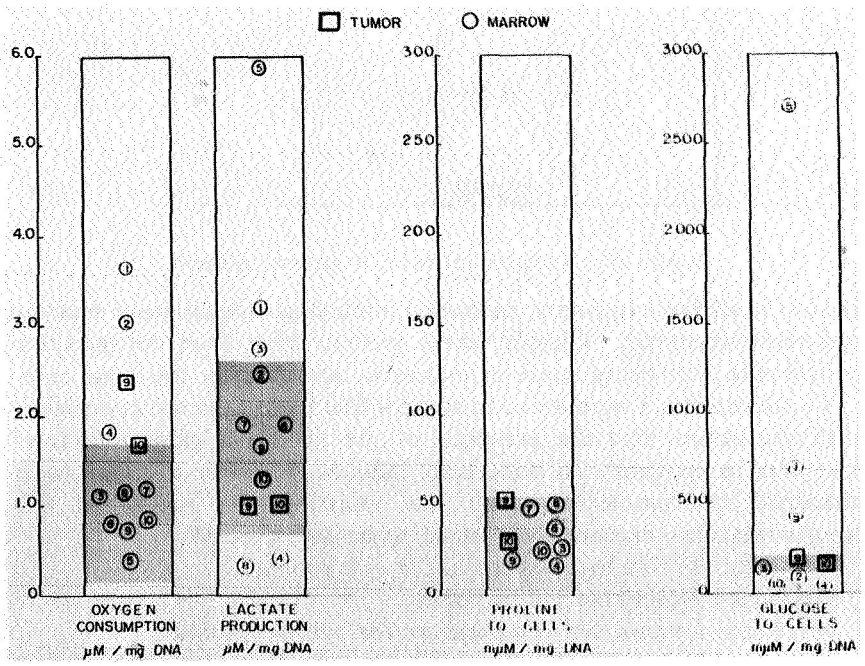

FIGURE 155. Marrow metabolism in multiple myeloma.

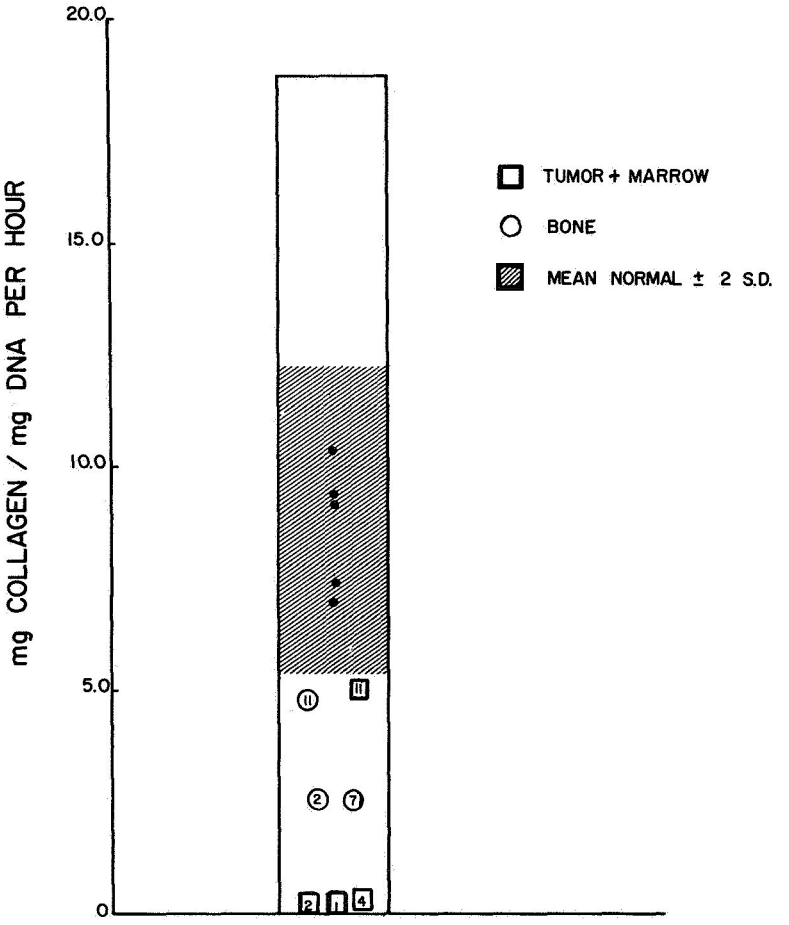

FIGURE 156. Collagenase activity in multiple myeloma.

or low in all the specimens examined, including myelomatous marrow and plasmacytomas. Clearly, some protein other than collagen and collagenase was being made in excessive amounts by the bone cells.

To summarize, I would like to suggest that these data are compatible with the notion that the presence of the abnormal plasma cells of myeloma in proximity to bone cells induces the latter to make some abnormal macromolecule. The real question—the nature of this macromolecule—remains, of course, to be investigated.

HOLTZER: Is that hydroxyproline or is it proline?

NICHOLS: Proline carried the label.

HOLTZER: I mean, did you measure for hydroxyproline?

NICHOLS: Not as such. The data are simply on incorporation of proline label without much more discrimination.

HOLTZER: Right.

NICHOLS: We have shown that the label in the collagen when derived from the proline is present in the proline and hydroxyproline. Is that what you mean?

HOLTZER: In that material, or in that protein, was there proline or was there hydroxyproline——

NICHOLS: In the cells? I do not know. I just have not looked.

SAXÉN: Can you exclude the possibility that your marrow samples were contaminated by the plasma cells?

NICHOLS: They contained plasma cells. The relative properties varied somewhat, but the data that are obtained from pure plasmacytoma are very similar, so that it probably does not matter.

HOLTZER: My question is, Is that making hydroxyproline-containing protein?

NICHOLS: I cannot answer that—I do not know.

HOLTZER: Then why do you say that the bone cells are making something? Why is it not just the myeloma cells that are making something?

NICHOLS: Because when you look at myeloma cells or myelomatous marrow, you find that the incorporation of proline into these cells is actually lower than the incorporation of proline into cells derived from the bone from which the marrow has been washed. So it seems unlikely that marrow-cell contaminants are responsible. It is not solid proof, of course.

BÉLANGER: Dr. Nichols, whenever we have a tumor anywhere, particularly a tumor of long duration and a tumor which would produce antigenic reactions in the organism, is it not possible that the system will react locally by producing cells that tend to destroy this tumor, and among those we would have, for instance, histiocytes? And if you have a large number of histiocytes in bone or in tumors or elsewhere, these are very well known to be rich in lysosomes, so they would increase, probably, the quantity of proteolytic enzymes which would be present in such tissue? This would be an important factor wherever you have tumor.

NICHOLS: This is perfectly true, and we have not measured other proteolytic enzymes besides collagenase so far. We think it would be amusing to see whether we could identify by electrophoresis or immunologic techniques a protein containing this excess label. Myeloma is of great advantage in this sort of work, since characteristic abnormal proteins are to be found in the plasma. Perhaps this is being made by bone cells operating under orders from the plasma cells.

PECK: The striking thing about myelomatous bone destruction is that it is unaccompanied by a response on the part of the bone that can be demonstrated histologically. Osteoblastic activity would seem

to be inhibited, and there is certainly no significant histiocytic response to support myeloma that can be demonstrated in these cavities.

I have a question just for the record. How many of these patients were receiving alkylating agents at the time of biopsy?

NICHOLS: The data on marrow (fig. 155) were from 11 patients, and of that group, if my memory serves me, there were two who were receiving alkylating agents, one or the other of the sarcolysins, at the time of biopsy. Some others had received sarcolysin. None of these patients was receiving fluoride at the time of biopsy. Since then, five have been on fluoride, of which we have biopsied four.

There are several intriguing things about the response of myeloma patients to fluoride. I do not have the faintest notion what they mean. I am sorry to say they may lend fuel to the fire of the idea that you must have proliferating tissue to get induction. Patients with multiple myeloma to whom one gives a therapeutic dose of fluoride develop fluorosis in a period of time which is much shorter than the time in normal subjects. It takes the normal subject, or the osteoporotic patient, at least 6 months to show a change in alkaline phosphatase and 9 to 12 months to show a roentgenographic change, whereas the myelomatous patients will show these changes in a matter of 6 to 8 weeks, including roentgenographic changes. We had one man who developed very marked widening of his trabecula in 2½ months.

PECK: To the best of my knowledge, the only cases that Dr. Cohen has published with respect to this response to fluoride were also receiving massive doses of androgens (ref. 229). Does this 6-week response occur in the absence of concomitant therapy?

NICHOLS: In the absence of androgen, yes. In the absence of calcium, we do not really know. All of these patients have been given calcium and some vitamin D, too. I do not know exactly how much of this is related to that response.

ARNAUD: Did any of them become hypercalcemic during the period of study?

NICHOLS: No. All of these patients were normocalcemic. It is interesting that all of the patients on fluoride have shown an inhibition of uptake of proline into the cells and a stimulation of proline into collagen, which is sort of a reversal of the normal pattern in our osteoporotic patients. In other words, the fluoride seems to return their bone metabolic pattern toward normal except for one man who, on the third biopsy taken in the final stages of his illness, showed a return to an abnormally active pattern after having previously shown the usual inhibition.

BAUER: I have recently reviewed some evidence, based on tracer studies with ^{47}Ca and ^{85}Sr, that myeloma does not elicit the intensive

repair reaction usually encountered in bone tissue hit by tumor, infection, or fracture (ref. 230).

NICHOLS: This is an important point, with relation to the biology of skeletal tissue at large, because one wonders whether we are seeing normal bone cells that have been induced to make some abnormal product. If this could be shown, then by what means? We may have a good example there of an induction system due to tumor. One also is tempted to ask, "Is the inducer that made the plasma cell abnormal the same one that made the bone cell abnormal?"

URIST: Would you like me to show you how to turn induction systems on or off?

MCLEAN: Yes.

URIST: This can be demonstrated by experiments with an implant of decalcified bone matrix (refs. 151 and 231). We have seen slides from some of our experiments on decalcified bone-matrix implants. Now I would like to summarize our findings of the chemical aspects of this process.

In collaboration with Barry Silverman, Juan de la Sierra, and other orthopedic research fellows at UCLA, we are testing decalcified bone treated or denatured in various ways by many different chemical agents. At this time, it can be reported that bone induction is inhibited if 0.6 *N* HCl-decalcified matrix is further denatured by: (1) boiling at 100° C, but not by heating, for 5 minutes at temperatures between 50° and 90° C; (2) incubation in solutions by pronase, ficin, papain, chymopapain, elastase, collagenase, but not by hyaluronidase, acid phosphatase, or alkaline phosphatase; treatment with β-propiolactone for sterilization, fluorodinitrophenol for blocking ϵ-amino and other reactive groups, or HNO_2 or HNO_3 for deamination of the tissue proteins, or metal ions with special toxic effects, e.g., $BeCl_2$.

Bone induction is either enhanced, or not perceptibly inhibited, by: (1) freezing at −30° C; (2) lyophilization at −70° C; (3) sterilization in 70 percent alcohol; (4) defatting with alcohol, acetone, ether, or detergents such as pHisohex; (5) extraction in concentrated salt solutions such as 0.5 *M* NaCl, or $CaCl_2$, to remove mucoproteins and lipoproteins; (6) treatment with solutions of various carboxylic acid blocking reagents such as toluidine blue, protamine sulfate, $PbCl_2$, $FeCl_3$, $CaCl_2$, and others.

These experiments demonstrate something about the influence of the chemical composition of the implant upon the cells that grow in and repopulate the interstices of the implant. They do not demonstrate whether chemical substances are transferred from the denatured matrix to the cytoplasm of the various cells that grow into the area of the implant. We performed an experiment with implants of 0.6 *N* HCl-

decalcified bone in a Millipore chamber, but observed no bone formation either inside or outside the Millipore membrane. Bone formed inside in one instance when the cell grew through a crack in the filter and gained contact with the matrix in one place. (See fig. 86(*b*).) Therefore, we may assume the inducer is mobilized by contact with cells.

SAXÉN: What kind of Millipore filter was it? Do you remember its pore size?

URIST: Pore size was 0.45 micron, 150 microns in thickness, the same as employed by Goldhaber (ref. 232).

To follow the pathways of proliferation of cells, ^{3}H-thymidine was injected into the host, and grain counts were made of labeled cells in various parts of the implant. The percentage of labeled cells was low in the area of histiocytes, the interstices of the old matrix, and very high in the progenitor cells around sprouting blood vessels, specifically in areas of active osteogenesis. In this respect, the picture resembled the distribution of labeled cells in the metaphysis of a growing bone.

To assemble our observations and describe the process in terms of modern concepts of embryonic induction, table XXXV illustrates a

TABLE XXXV

BONE AUTOINDUCTION: CELL-INDUCTION SEQUENCES

Days after implantation	Before induction: Inducing cell	Before induction: Responding cell	Inducing matrix and microenvironmental variables	After induction: Responding cell	After induction: Differentiated cell
1 to 22	A	B	+ (CO_2 tension, O_2 saturation, Cell metabolites)	B	D
	Wandering histiocyte	Fixed histiocyte	+ (Open excavation chamber, High O_2 saturation, Low CO_2 tension, Other cell metabolic products)	Fixed histiocyte	Osteoprogenitor cell and osteoblast
22 to 30	A	B	+ (CO_2 and O_2 saturation, Cell metabolites)	B	C
	Wandering histiocyte	Fixed histiocyte	+ (Compaction of cells in closed vascular channel, High CO_2 tension, Low O_2 saturation, Other cell metabolic products)	Fixed histiocyte	Chondroprogenitor cell and chondroblast
22 to 30	C	B	+ (Vascularized excavation chamber)	B	D
	Chondrocyte	Perivascular connective tissue cell	+ (Absorption cavities, High CO_2 tension, Low O_2 saturation)	Perivascular connective tissue cell	Osteoprogenitor cell and osteoblast

Adapted from ref. 151.

theory of autoinduction in which: (1) the decalcifying matrix is an inducing surface; (2) the progeny of the first generation of cells to populate the implant are the inducing cells; (3) young perivascular connective tissue cells are the responding cells; (4) progenitor cells are the induced cells; and (5) cell specialization for cartilage formation and for bone formation is the culmination of cell migration, a series of mitotic divisions, and the product of the interaction of the cell and its microenvironment. Osteogenesis is, therefore, not a triggered event, but depends upon a sequence of cell changes which occurs in a bone-matrix implant over a period of 3 weeks. The surface of the inducing matrix could affect the cell membrane, transfer material to the ribosomal system or the genetic structure, but we have no information at this time either to implicate or reject a reaction at any one of the three sites in the cell. Osteogenesis developed in this way should be regarded as autoinduction insofar as both inducing and responding cells originate in the same individual.

Neither the nature of the inducer nor the local chemical mechanisms of induction is known, but present investigations promise to produce an important advance in knowledge of bone physiology. The experiments mentioned deal chiefly with inducing surfaces of the matrix. Our work at present also deals with systemic factors such as the competence of the responding cells. We are now finding, for example, that an aged host produces relatively little bone induction and always after some delay. Antimetabolites, antibiotics, and other cell toxins are being tested to determine the action of various ultramicroscopic organelles in bone induction.

PRITCHARD: That was very nicely told, Dr. Urist.

URIST: Thank you. If there are no further comments, we will now adjourn this meeting.

ROBINSON: Before we go, I would like to thank Dr. Fremont-Smith and the New York Academy of Sciences for a very pleasant meeting here, and particularly Dr. Urist and the others who have given us useful information.